HLA-A, B, C, DRB1, DQB1 matched siblings (29), matched unrelated donors (22), or unrelated donors mismatched for one HLA antigen (6), homozygous mismatch (1), one HLA allele (2), or two HLA alleles (1). Fludarabine 40 mg/m^2 was given intravenously daily for four days, with each infusion followed immediately by intravenous busulfan. The dose of busulfan for days 1 and 2 was 130 mg/m^2. Pharmacokinetic analysis was performed after the first infusion of busulfan; in 59 pts, the goal was to adjust busulfan doses for days 3 and 4 to achieve an average targeted Css level of 800-1000 ng/ml. Levels were drawn incorrectly in 4 of these pts and doses were not changed. Thirty-five (59%) pts had their doses adjusted, increased in 27 and decreased in 8, while 20 pts had Css within the desired range without adjustment. Patients received tacrolimus and standard doses of methotrexate for GVHD prophylaxis, with the exception of five patients. Engraftment occurred in 58 (95%) pts. Thirty (64%) of 47 pts followed for at least 100 days experienced acute GVHD requiring treatment. Six pts have died of transplant-related complications and 7 pts have failed to achieve remission or have relapsed. Median follow-up is 174 days (range 26-448 days). The 100-day K-M estimate of survival for the whole cohort is 92%, and event-free survival 88%. The 100-day mortality in this study compares well with the 100-day mortality reported to the IBMTR for patients with AML, ALL, MDS, and CML transplanted from either HLA-matched siblings or unrelated donors. These preliminary results indicate that tBuFlu is a promising myeloablative regimen that can be utilized in older patients with low early treatment-related mortality.

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LACK OF IMMUNE BARRIER TO ALLOGENEIC HEMATOPOIETIC STEM CELL ENGRAFTMENT IN T, B, AND NK CELL DEFICIENT (RAG2ΓC$^{-/-}$B6) MICE

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The way that allogeneic hematopoietic stem cells (HSC) resist engraftment is not completely understood. Natural killer cells (NK) and lymphocytes are thought to mediate the allograft barrier in mice that are mismatched at the major histocompatibility complex (MHC). The clearing of niche space is also thought to be required for donor cell engraftment. Here we attempt to dissect the relative contribution of these host elements to hematopoietic resistance using genetically defective Rag2$^{-/-}$ (*H2^b*) mice lacking T and B cells, or Rag2γc$^{-/-}$ (*H2^b*) mice lacking in T, B, and NK cells as recipients. We have previously shown that HSCs encounter greater resistance to engraftment when compared to unfractionated bone marrow (BM), and the resistance can be quantitated by titrating numbers of HSCs needed to rescue lethally irradiated recipients. Rescue of syngeneic or CD45 congenic recipients requires only 200 HSCs, whereas higher HSC doses are required as the genetic disparity increases. In this study, radioresistant MHC-mismatched AKR/J (*H2^k*) HSCs were transplanted into lethally irradiated (950 cGy) B6.WT (*H2^b*). All B6.WT mice died of hematopoietic failure despite attempted rescue with 1000 AKR/J HSC. No significant improvement in engraftment was observed in Rag2$^{-/-}$ mice when compared to B6.WT mice. However, an impressive difference was noted in the Rag2γc$^{-/-}$ mice, in which the immune barrier completely disappeared. A dose of 300 HSC rescued all irradiated Rag2γc$^{-/-}$ mice and even 200 AKR HSC, an amount equivalent to a congenic dose rescued 100% of recipients. We then sought to determine if engraftment could be achieved using non-myeloablative conditioning, or no radiation at all. Rag2γc$^{-/-}$ recipients of 6000 AKR/J HSCs treated with 500 cGy-300 cGy resulted in 100% donor engraftment. Additionally, unconditioned Rag2γc$^{-/-}$ also engrafted since 10-20% of donor AKR/J granulocytes were detected. In contrast, unconditioned Rag2$^{-/-}$ mice showed no evidence of donor cell engraftment. We also studied the trafficking of allogeneic FVB (H-2^q) HSC in irradiated versus unirradiated Rag2γc$^{-/-}$ (H-2^d) recipients by in vivo bioluminescence imaging. HSC were observed to enter the marrow space of irradiated mice within minutes following infusion, whereas unirradiated mice demonstrated no luciferase signal until day +5 post-infusion. We conclude that Rag2γc$^{-/-}$ mice have a profound reduction in the immune barrier to allogeneic HSC engraftment and, that in irradiated mice, HSC rapidly enter the marrow.

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POST-TRANSPLANTATION CYCLOPHOSPHAMIDE (Cy) AS A SINGLE AGENT FOR GVHD PROPHYLAXIS AFTER HLA MATCHED RELATED AND UNRELATED BONE MARROW TRANSPLANTATION

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In animal models of BMT, a properly timed high dose of Cy post-BMT selectively eliminates host-versus-graft and graft-versus-host reactive T cells, thereby preventing graft rejection and reducing GVHD. We hypothesized that high dose posttransplant Cy (50 mg/kg IV) administered on days +3 and +4 after BuCy conditioning may be effective in preventing GVHD and can limit, or entirely eliminate the need for, standard postgrafting immunosuppression. This should lessen immunosuppression and allow early institution of additional posttransplant immunotherapy such as DLI. 28 patients with advanced hematologic malignancies were conditioned with busulfan (PO or IV) on days −7 to 3 and Cy on days −2 and −1, transplanted with non–T cell–depleted marrow, and treated with Cy on days +3 and +4 as only postgrafting immunosuppression. 15 patients (median age 41 years) were allografted with bone marrow from HLA-identical siblings. Time to neutrophil (>500/μl) and platelet (>20000/μl, untransfused) engraftment was 22 and 31 days, respectively. One patient experienced secondary graft failure and was successfully rescued. Acute GVHD occurred in 7/15 patients at a median of 43 days after transplantation (range 20-68 days) and was exclusively grades I (2 patients) and II (5 patients). All 7 patients with GVHD responded completely to standard therapy (steroids only or steroids + FK-506) and all of them were successfully rapidly weaned from all immunosuppressive agents. With a median follow-up of 290 days (range 50-380), 10/15 patients are alive (all 5 patients died of relapsed disease) of which 7 are in remission. 13 patients (median age 41 years) received bone marrow from HLA-matched unrelated donors. Primary graft failure occurred in 2 recipients of unrelated marrow, and was fatal in one. One patient died from VOD. Time to neutrophil and platelet engraftment was 25 and 71 days, respectively. Of the 11 patients that engrafted, 1 developed grade I, 4 developed grade II and 1 developed grade III acute GVHD. All of them rapidly responded to standard therapy. From an overall survival perspective, 10/13 patients are alive of which 6 are in remission, with a median follow-up of 290 days (range 75-430). This preliminary analysis suggests that high dose post-transplantation Cy is effective as a single agent in the prophylaxis of severe GVHD after myeloablative conditioning and HLA-matched related BMT and should be studied in patients with standard risk hematologic malignancies.

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OUTCOME OF ALTERNATIVE DONOR TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA CAN BE COMPARABLE TO OUTCOME WITH MATCHED RELATED DONORS

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Matched related donor (MRD) bone marrow transplantation is the treatment of choice for pediatric patients with severe aplastic anemia (SAA); however, only 25% of patients will have an HLA-identical sibling. Alternative donor transplants may be an option for these patients, but such therapies have been associated with greater incidences of graft failures and graft-versus-host disease (GVHD). We retrospectively analyzed 32 pediatric patients who have undergone 34 hematopoietic stem cell transplants for severe aplastic anemia at our institution from April 1997 to April 2005. One patient had a MRD transplant followed by a matched unrelated donor (MUD) transplant, while another had an HLA-mis-